3-Amino-3,4-dihydro-2*H*-1-benzopyran Derivatives as 5-HT$_{1A}$ Receptor Ligands and Potential Anxiolytic Agents. 2. Synthesis and Quantitative Structure−Activity Relationship Studies of Spiro[pyrrolidine- and piperidine-2,3′(2′*H*)-benzopyrans]†

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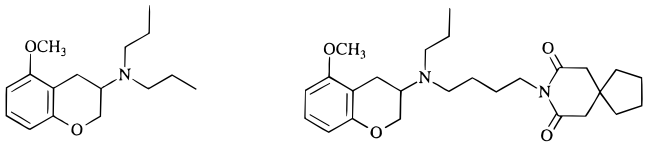
In continuation of our work on 3-amino-3,4-dihydro-2*H*-1-benzopyran derivatives with high affinity for 5-HT$_{1A}$ receptors, we have prepared rigid spirobenzopyran analogues designed from the pharmacophore models of Mellin and selected *via* a quantitative structure−activity relationship approach mainly based on similarity indices. A series of spiro[pyrrolidine- and piperidine-2,3′(2′*H*)-benzopyrans] with various substitutions on the aromatic ring as well as on the extracyclic spiranic nitrogen atom were then synthesized and evaluated for their serotonergic and dopaminergic activities. Good correlation between the predicted and the experimental binding values was observed with an average difference of 0.2 unit on log(IC_{50}). Affinities for the 5-HT$_{1A}$ receptors were in the nanomolar range for the best compounds ((+)-**11a**, **23**) with a high selectivity versus other 5-HT (5-HT$_{1B}$, 5-HT$_2$, 5-HT$_3$) or dopamine (D_1, D_2) receptor subtypes. As for the 3-amino-3,4-dihydro-2*H*-1-benzopyran series, the dextrorotatory enantiomer (+)-**11a** showed better affinity and selectivity for 5-HT$_{1A}$ receptors than its levorotatory analogue (−)-**11a**. Compound (+)-**11a** proved *in vitro* to be a full agonist and *in vivo* to be active in various comportmental tests predictive of psychotropic activity, such as the forced swim test and the tail suspension test, and is currently under complementary investigations.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) and its receptors are involved in many physiological or pathophysiological processes such as sleeping, thermoregulation, noxious effects, feeding, sexual behavior, and depression, migraine, anxiety, and Alzheimer's disease.[1−7] Many serotonin receptor subtypes have become known and classified into four main families: 5-HT$_1$, 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$,[8,9] although others such as 5-HT$_5$, 5-HT$_6$, and 5-HT$_7$[10] have been identified from cloning studies.[11−13] The heterogeneous 5-HT$_1$ class was subdivided into six accepted subtypes: 5-HT$_{1A}$, 5-HT$_{1B}$,[14] and, more recently, 5-HT$_{1D\alpha}$, 5-HT$_{1D\beta}$,[15] 5-HT$_{1E}$,[16] and 5-HT$_{1F}$[17] (the previously called 5-HT$_{1C}$ receptors[18] were renamed 5-HT$_{2C}$).[17] The role of these receptor subtypes in physiological or pathophysiological states and their respective agonists, partial agonists, and antagonists are reported in several recent reviews.[19−21] It is generally accepted that serotonin receptors of the 5-HT$_{1A}$ subtype are involved in psychiatric disorders like depression and anxiety.

Many compounds of different chemical classes (indoles, aminotetralines, arylpiperazines, benzodioxanes) have a high affinity for 5-HT$_{1A}$ receptors[8,22−25] and may act as agonists, partial agonists, or antagonists. Buspirone, an arylpiperazine derivative, was the first agent to be approved for clinical use as an antianxiety agent.[26]

Chart 1



1 **2**

Our interest in the development of such therapeutic agents that display high affinity for 5-HT$_{1A}$ receptor sites has recently led us to synthesize several derivatives of 3-amino-3,4-dihydro-2*H*-1-benzopyran, for example, compounds **1** and **2** (Chart 1).[27−31] One of these, (+)-8-[4-[*N*-propyl-*N*-(5-methoxy-3,4-dihydro-2*H*-1-benzopyran-3-yl)amino]butyl]-8-azaspiro[4.5]decane-7,9-dione (S20499, (+)-**2**), is today under development.[31] Modeling studies performed from the pharmacophore models of Mellin *et al.*[32] with our 3-amino-3,4-dihydro-2*H*-benzopyran derivatives have shown that it might be possible to obtain 5-HT$_{1A}$ ligands with high affinity and selectivity by "freezing" them in a conformation capable of improving interactions with the receptor. The only way to obtain such results is to rigidify the molecule's structure in the appropriate conformation, but this approach is mainly qualitative and has a limited predictive power. We have therefore used a quantitative structure−activity relationship (QSAR) approach, mainly based on the similarity indices, to get a mathematical model with a very good predictive power

† Part 1 is described in ref 31.
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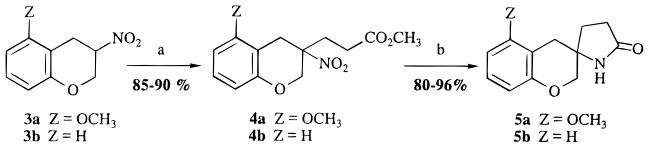
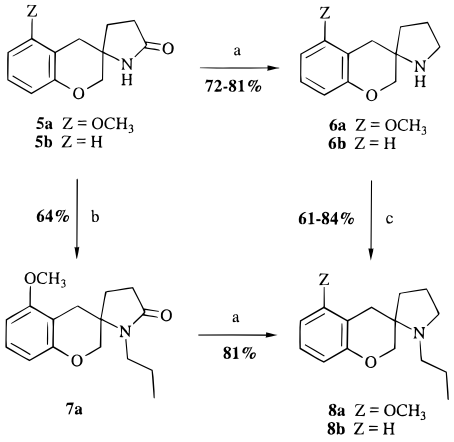
Scheme 1[a]



[a] (a) CH_2=$CHCO_2CH_3$, $PhCH_2N^+(CH_3)_3$, MeO^-, MeOH; (b) H_2, Raney Ni, MeOH.

Scheme 2[a]



[a] (a) (i) BH_3·Me_2S, THF, (ii) HCl, MeOH; (b) *n*-PrI, NaH, DMF; (c) *n*-PrI, K_2CO_3, DMF.

within this series. The synthesis, QSAR results, receptor binding profile, and behavioral activity of these compounds (analogues of S20499) which possess the aminobenzopyran moiety with an extracyclic nitrogen in the spiranic system and with various substitutions on the aromatic ring as well as on the extracyclic nitrogen atom are reported in this paper.

Chemistry

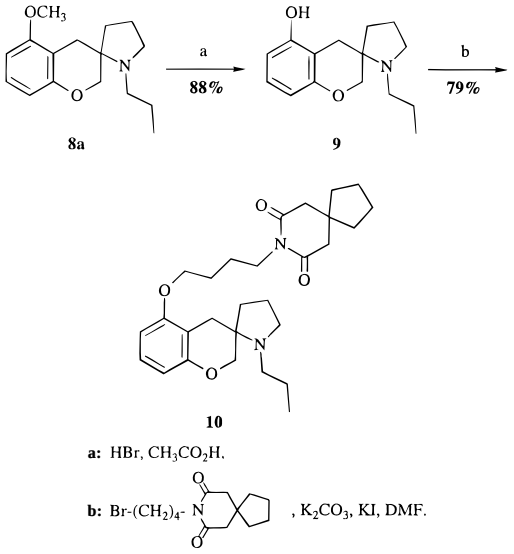
The Michael condensation[33] of 3-nitro-3,4-dihydro-2*H*-1-benzopyrans **3**[31,34] with methyl acrylate in the presence of benzyltrimethylammonium methoxide afforded the nitro ester derivatives **4**. Compounds **4** were reductively cyclized with Raney nickel in methanol to give 5-oxospiro[pyrrolidine-2,3′(2′*H*)-benzopyran] derivatives **5** (Scheme 1).

Reduction of amides **5** with borane−dimethyl sulfide complex in tetrahydrofuran[35] afforded spiro[pyrrolidine-2,3′(2′*H*)-benzopyran] derivatives **6** in 72−81% yields. *N*-Propylated amines **8** were obtained in good yields by *N*-alkylation of **6** with 1-iodopropane in *N*,*N*-dimethylformamide in the presence of potassium carbonate. *N*-Propylation of amide **5a** was carried out in *N*,*N*-dimethylformamide in the presence of sodium hydride and 1-iodopropane to give **7a** in 64% yield. Reduction of amide **7a** by the procedure described above, utilizing borane−dimethyl sulfide complex, afforded amine **8a** in 81% yield (Scheme 2).

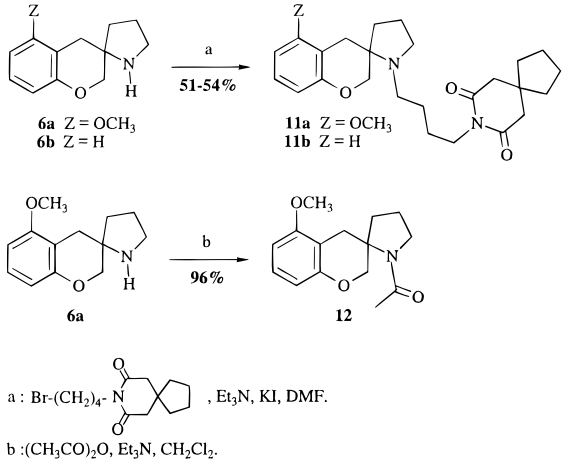
O-Demethylation of **8a** was realized with hydrobromic acid in refluxing acetic acid[36] to give **9** in 88% yield. The *O*-alkylated derivative **10** was easily obtained by treatment of **9** with 8-(4-bromobutyl)-8-azaspiro[4.5]decane-7,9-dione in *N*,*N*-dimethylformamide in the presence of potassium carbonate (Scheme 3).

Compounds **11** were prepared by alkylation of corresponding secondary amines **6** with 8-(4-bromobutyl)-8-azaspiro[4.5]decane-7,9-dione in *N*,*N*-dimethylformamide as solvent in the presence of triethylamine.

Scheme 3



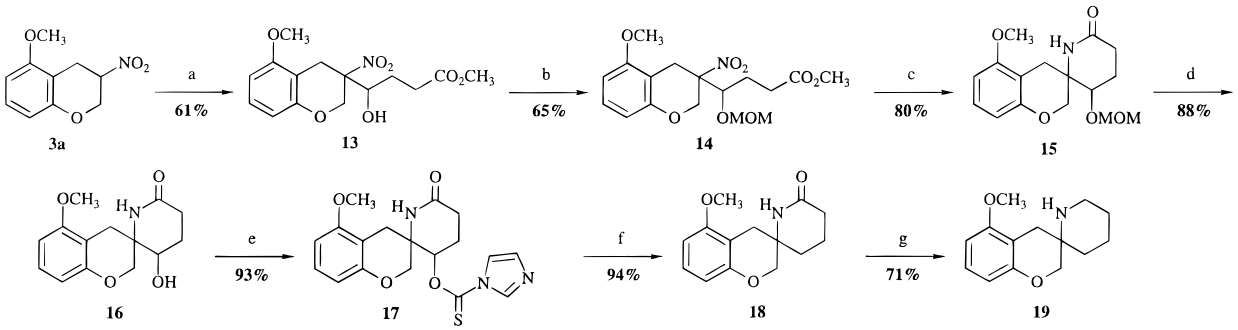
Scheme 4



Treatment of **6a** with acetic anhydride in the presence of triethylamine gave amide **12** in 96% yield (Scheme 4). The separation of the enantiomers of compound **6a** was achieved by enantioselective crystallization with optically pure 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate as chiral resolving agent.[37] Enantiomeric purity was determined by using HPLC techniques (cf. the Experimental Section). The compounds (+)-**11a** and (−)-**11a** were respectively prepared by alkylation of secondary amines (+)-**6a** and (−)-**6a** with 8-(4-bromobutyl)-8-azaspiro[4.5]decane-7,9-dione in the presence of triethylamine.

The second target of our studies aimed to prepare the 1-substituted-5′-methoxy-3′,4′-dihydrospiro[piperidine-2,3′(2′*H*)-benzopyran] derivatives **22**−**25**. As shown in Scheme 5, 5-methoxy-3-nitro-3,4-dihydro-2*H*-1-benzopyran (**3a**)[34] was treated by *n*-butyllithium in tetrahydrofuran as solvent and methyl-4-oxobutanoate as electrophile agent to give the desired nitro alcohol **13** in 61% yield.[38] Reduction of **13** by catalytic hydrogenation led to a retroaldolization reaction instead of the expected cyclization. To avoid this reaction, the protection of the hydroxy group of **13** was necessary. The *O*-alkylation of **13** in chloroform by chloromethyl methyl ether in the presence of diisopropylethylamine generated the desired compound **14** in 65% yield.[39] Reduction of **14**, by hydrogenation in the presence of Raney nickel in

Scheme 5



a : (i) BuLi, THF (ii) *t*-Bu(CH$_3$)$_2$SiCl, THF (iii) OHC(CH$_2$)$_2$CO$_2$CH$_3$, Bu$_4$NF, THF.

b : MOMCl, *i*Pr$_2$NEt, CHCl$_3$. c : H$_2$, Raney Ni, MeOH. d : Amberlyst 15 (ion exchange resin), MeOH.

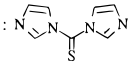
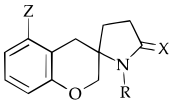
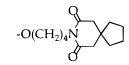
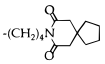
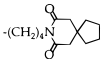
e :  , THF. f : *n*-Bu$_3$SnH, AIBN, PhCH$_3$. g : (i)BH$_3$.Me$_2$S, THF (ii) HCl, MeOH.

Table 1. Physical Data for 1-Substituted-3′,4′-dihydrospiro-[pyrrolidine-2,3′(2′*H*)-benzopyran] Derivatives **5**−**12**



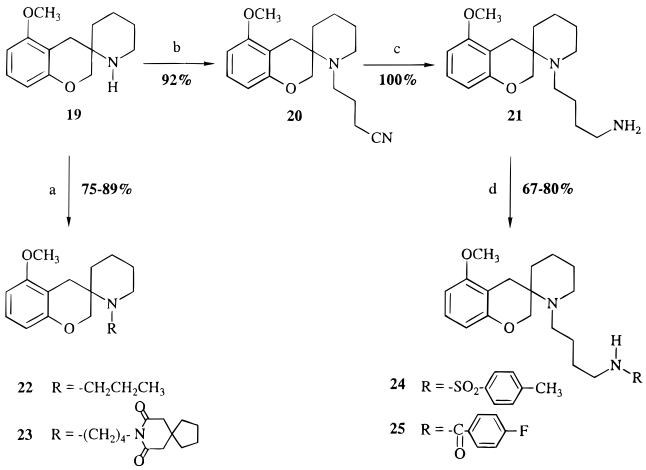
Compds	Z	R	X	Yield, %[a]	mp, °C	Formula
5a	OCH$_3$	H	O	91	210-211	$C_{13}H_{15}NO_3$
5b	H	H	O	96	179-180	$C_{12}H_{13}NO_2$
6a	OCH$_3$	H	H$_2$	76	oil	$C_{13}H_{17}NO_2$
6b	H	H	H$_2$	72	oil	$C_{12}H_{15}NO$
7a	OCH$_3$	C_3H_7	O	64	89-90	$C_{16}H_{21}NO_3$
8a	OCH$_3$	C_3H_7	H$_2$	84	oil	$C_{16}H_{23}NO_2$
8b	H	C_3H_7	H$_2$	61	oil	$C_{15}H_{21}NO$
9	OH	C_3H_7	H$_2$	88	185-186	$C_{15}H_{21}NO_2$
10		C_3H_7	H$_2$	79	oil	$C_{28}H_{40}N_2O_4$
11a	OCH$_3$		H$_2$	54	oil	$C_{26}H_{36}N_2O_4$
11b	H		H$_2$	55	oil	$C_{25}H_{34}N_2O_3$
12	OCH$_3$		H$_2$	96	120-121	$C_{15}H_{19}NO_3$

[a] Yields of isolated products based on compounds **4**−**9**, after purification by column chromatography.

methanol as solvent, afforded the piperidinone **15** in 80% yield. Deprotection of the hydroxy group was carried out in methanol in the presence of Amberlyst 15, ion-exchange resin (Aldrich), to give the desired alcohol **16** in 88% yield. Deoxygenation of **16** was realized in two steps. At first, the thiocarbonyl derivative **17** was prepared from alcohol **16** and *N*,*N*′-thiocarbonyldiimidazole in tetrahydrofuran in excellent yield (93%), and then, the treatment of **17** by tributyltin hydride in toluene in the presence of 2,2′-azobis(2-methylpropionitrile) afforded the expected derivative **18**.[40] Reduction of amide **18** with borane−dimethyl sulfide complex in tetrahydrofuran gave in 71% yield the desired amine **19** (Scheme 5).[35]

N-Substituted amines **22** and **23** were obtained in good yields (75−89%) by *N*-alkylation of **19** with the appropriate halogeno derivatives and potassium carbonate in acetonitrile as solvent in the presence of potassium iodide (Scheme 6). Treatment of **19** with 4-bromobutyronitrile and potassium carbonate in *N*,*N*′-dimethylformamide and in the presence of potassium iodide gave the cyano derivative **20** in 92% yield. Reduction of **20** using lithium aluminum hydride in ether gave the amine **21** in quantitative yield. Condensation of this amine **21** with *p*-toluenesulfonyl chloride or 4-fluorobenzoyl chloride gave the expected sulfonamide **24** and amide **25** in 80% and 67% yields, respectively (Scheme 6).

Scheme 6



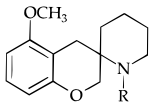
a : RX (X=I, Br), K_2CO_3, KI, CH_3CN. b : Br(CH$_2$)$_3$CN, K_2CO_3, KI, DMF.

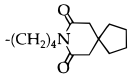
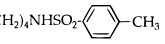
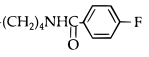
c : $LiAlH_4$, Et_2O d : RCl, Et_3N, CH_2Cl_2.

Molecular Modeling

QSAR has provided a valuable aid in developing new therapeutic agents. It is generally thought that noncovalent forces dominate receptor−drug interactions and that these forces can be described in terms of steric and electrostatic effects. In an attempt to relate these effects to observed biological data, a number of approaches have been presented.[22,41] All the products of this paper were first evaluated using the pharmacophore models[25,32] of 5-HT$_{1A}$ ligands described previously. Only the putative compounds presenting a good fit with this pharmacophore have been synthesized. However this approach is only qualitative. It has recently been shown that good quantitative structure−activity relationships can be obtained through a statistical analysis of molecular similarity matrices.[42,43] We have used this

Table 2. Physical Data for 1-Substituted-5′-methoxy-3′,4′-dihydrospiro[piperidine-2,3′(2′*H*)-benzopyran] Derivatives **22**−**25**



Compds	R	Yield, %[a]	mp, °C	Formula
22	-C_3H_7	75	oil	$C_{17}H_{25}NO_2$
23		89	oil	$C_{27}H_{38}N_2O_4$
24		80	oil	$C_{25}H_{34}N_2O_4S$
25		67	oil	$C_{25}H_{31}N_2O_3F$

[a] Yields of isolated products based on amine **19** or **21**, after purification by column chromatography.

approach on a set of 52 compounds presented in this paper and previous ones,[30,31,44] which were tested for 5-HT_{1A} receptor binding. The training set was made from the 48 products for which we had the binding results on the 5-HT_{1A} receptor. We have used these relationships to predict the affinity of new compounds not yet synthesized at the time of the calculations. The results for four compounds (**22**−**25**) are presented here.

Methods of Calculation. The structures of the chromans, incorporated into the QSAR analysis, were constructed using the molecular modeling package from Oxford Molecular.[45] For this modelization study, the molecules were used in their neutral form, although, at physiological pH, they are protonated. The initial structures were generated using COBRA[45] V3.0. A conformational analysis was made using an exhaustive search and the COSMIC[46] force field. We have retained, for our studies, all the conformers within a 10 kcal/mol range from the minimum value. We then checked among these conformers for the presence of at least one which would have a good fit with the pharmacophore model of Mellin *et al.*[32] and no atom in the excluded volume. The superposition was made using the aromatic ring of the benzopyran moiety and a dummy atom nitrogen vector (the basic nitrogen) according to the geometry of Mellin. For the following work, we retained for each product the conformer of lowest energy having a good fit with the pharmacophore. The partial charges (partial net charges coming from the diagonal of the density matrix) of this conformer were calculated with MOPAC[47] V5.0 (keywords: AM1, precise). Most of the binding results (for the compounds with a chiral center) were obtained from the racemic mixtures. For these compounds, we have arbitrarily chosen only to use the *R*-isomers in this study.

Fit of Compounds with Mellin Model. Using the fitting method described previously, we have superposed the 16 products of this paper, tested for the 5-HT_{1A} receptor, to the pharmacophore model. The calculation of the root mean squares (rms) was made using the nitrogen atom, the end point of the lone electron pair, the center of the aromatic ring, and a dummy atom situated at ≈2.35 from the center of the ring on a perpendicular to the plane of this ring. For each compound, we found a conformer with a rms lower than ≈0.1 and without steric interactions with the described excluded volume. An example of this good fit is given in Figure 1 for the compound **11a**. The main limitation of this approach is its qualitative aspect. These compounds with a good fit have a range of binding to the 5-HT_{1A} receptor from about 10^{-4} to 10^{-10} M. We then tried to use a more quantitative method.

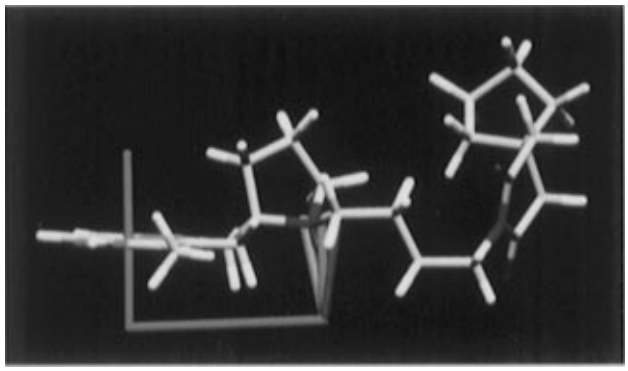


Figure 1. View of the superposition of compound **11a** and the Mellin model.

Calculation of Similarity Indices. Numerical indices which measure the overall electrostatic (or lipophilic) potential (or field) and the steric similarity between pairs of molecules have been used previously in affinity data correlations.[48] The most commonly applied formula for the calculation of molecular similarity is the Carbo index.[49] Molecular similarity is determined from the structural properties of the two molecules being compared. For this investigation electrostatic (and lipophilic) potential, electrostatic (and lipophilic) field, and shape have been calculated with the ASP V3.0 program.[45] For the generation of the similarity indices the common part of the compounds (unsaturated and saturated cycles of chromans) were aligned by a least-squares fitting. The calculation was then made with full optimization: The position of the lead molecule is fixed, the molecule, whose similarity index is calculated, is translated and rotated, and all its torsional bonds are also rotated in order to optimize the similarity index.

Electrostatic properties are calculated using, for each atom, the charges computed with MOPAC. For lipophilic properties the fragmental constants of Ghose[50] are used. For each of these two properties, field and potential are calculated. Briefly potential $P(r)$ in a point is given by

$$P(r) = \sum_{j=1}^{n} \frac{q_j}{|r - r_j|}$$

and field $F(r)$ by

$$F(r) = \sum_{j=1}^{n} \frac{q_j(r - r_j)}{|r - r_j|^3}$$

where r is the position of the point, q_j is the property in the atom j (charge or lipophilic contribution), r_j is the position of this atom, and n is the number of atoms in the molecule. The potential then gives an idea of of the location and the strength of ionic interactions; the field gives the similar information for dipolar interactions.

Table 3. Statistical Data for Similarity Calculations

evaluation methods	results[a]
electrostatic potential	0.82/0.46/7
lipophilic potential	0.76/0.32/8
electrostatic field	0.89/0.45/6
lipophilic field	0.83/0.39/6
shape	0.73/0.30/5

[a] The first value shows the r^2, the second value is the cross-validation $r(CV)^2$, and the third value shows the associated number of PLS components for the full matrix.

Shape similarity indices are evaluated in a similar manner using a modified version of the Carbo equation proposed by Meyer.[51]

For the potentials and the shape, we have used the analytical method of Good *et al.*[52] For the potentials this technique fits the inverse distance dependence to an expansion of Gaussian functions. For the shape the atomic electronic density functions are used instead of the van der Waals radius. The atomic orbitals wave functions squared are fitted with an expansion of Gaussian functions. This results in an increased computational speed. The number of Gaussian terms depends on the accuracy required. In all the calculations we have used three Gaussian terms. For the fields we have used a grid approach. The box is created using an extension of ≈4 beyond the limits of the combined volumes of the molecules. The distance between each point of the grid is ≈1. The points which are inside the van der Waals volume of the molecules are excluded from the calculations.

Results of QSAR Analysis. Using the similarity indices approach described above, we obtained five tables of descriptors (52 rows × 48 columns). Each table is made of a type of similarity indice (shape, electrostatic field or potential,..). Each row corresponds to a compound of either the training set or the set of new compounds to be predicted; each column corresponds to a compound of the training set. This training set was made of 12 compounds of this paper (**1**, **2**, **5a**, **6a**, **7a**, **8a**, **8b**, **9**, **10**, **11a**, **11b**, **12**) for which we had the binding data at the time of the study and 36 other compounds of the laboratory.[30,31,44] All these compounds have the same benzopyranic moiety. The entire list of compounds (training set (Table A), set of new compounds (Table B)), the five tables (Tables C−G) of similarity indices, and the results of the predictions (Table H) are available as Supporting Information.

The relationship between biological affinity and descriptors was analyzed using the partial least squares[53] (PLS) module of the TSAR[45] V2.1 program. For a compound, its similarity indices with the 48 compounds of the training set were the independent variables and its binding value was the dependent variable. Leave-one-out cross-validation has been used for all evaluation methods. For each PLS analysis we have retained the number of components which correspond to the first maximum value of the cross-validation r(CV)2.

Discussion. Table 3 shows the results of the five evaluation methods for the training set of compounds. It is clear that the best methods are the two electrostatic approaches (field and potential) which respectively are made of six and seven PLS components. The correlations between calculated and experimental binding values are represented for these two models in Figures 2 and 3. We have also calculated the mean of the two

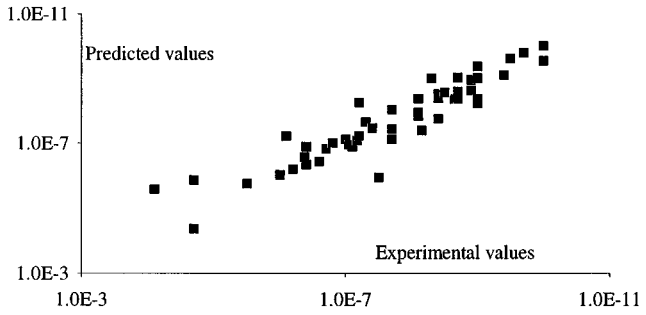


Figure 2. Correlation between experimental values and predicted values for electrostatic potential.

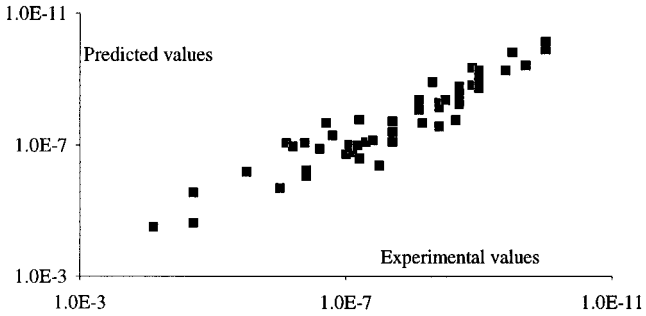


Figure 3. Correlation between experimental values and predicted values for electrostatic field.

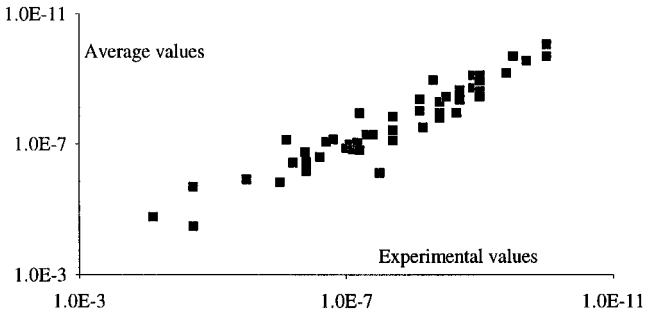


Figure 4. Correlation between experimental values and mean values.

methods. It does not change significantly the statistical coefficients, but the choice of the mean decreases the probability of outliers when using this method in a predictive aim. The correlation between experimental values and this new value is given in Figure 4. The table (Table H) with the entire set of data is available as Supporting Information. A closer examination of the correlation between the experimental and predicted values in Figures 2−4 reveals a better correlation for the high values of binding than for the low ones. The average difference for the 10 compounds with the lowest value is 0.46 unit of $-\log(IC_{50})$; for the 10 compounds with highest values it is 0.17 unit of $-\log(IC_{50})$. This phenomena seems to be normal in such QSAR studies, the classical explanation being the fact that there is only one (or a very few) possibility for a good binding but there are many reasons for a bad one (not only bad electrostatic distribution but also problems of lipophilicity and shape, for example). From the actual knowledge of the receptor, we can explain the choice of the electrostatic descriptors. The classical three-point model has a lipophilic site (the aromatic ring)[54] which is present in all the compounds and also two electrostatic sites. The main differences in this homogeneous set are the electronic distributions induced by the various

Table 4. Results of Prediction Calculations

	predicted values			
compd	electrostatic potential	electrostatic field	exptl values	mean values
22	2.6×10^{-7}	0.9×10^{-7}	4.2×10^{-7}	1.8×10^{-7}
23	10.7×10^{-9}	8.1×10^{-9}	8.8×10^{-9}	9.4×10^{-9}
24	8.1×10^{-8}	10.0×10^{-8}	6.8×10^{-8}	9.1×10^{-8}
25	5.8×10^{-8}	25.1×10^{-8}	6.3×10^{-8}	15.4×10^{-8}

substituents. The differences in shape are mainly located on a flexible part of the structure. Therefore their role is minimized.

We have used these three models (electrostatic field and potential and their mean) to design new ligands. In this paper we only present the results for four compounds which have been synthesized after the results of the modelization. Table 4 gives the experimental results of binding and the predictions of the three models. The correlation between the predicted and the real values is quite good. The values predicted by either the field or the potential were similar for three compounds (**22**−**24**). The experimental result is found to be very close to these values (and obviously of their mean). For compound **25** the two methods of prediction differed by 0.6 unit of $-\log(IC_{50})$. The use of the mean value allows in that case a better confidence in the result. After the synthesis and the binding tests of this product, the experimental result has been found very similar to the prediction of the model made with the electrostatic potential. However it was not possible to make a choice between the two predictions before its synthesis.

For these four compounds, the difference between the mean of predictions and the experimental value, within a range of 1.8 units, is 0.2 unit of $-\log(IC_{50})$. This difference is similar to the one found in the training set for the compounds with the highest values of affinity. This is another proof of the validity of these models. Their predictive power justifies the interest in such an approach which is, by nature, complementary to the pharmacology approach. Its function is mainly to predict a quantitative value and not to be the automated design for new molecules.

Results and Discussions

Sixteen 3′,4′-dihydrospiro[pyrrolidine- or piperidine-2,3′(2′*H*)-benzopyran] derivatives were designed, prepared, and firstly evaluated for both their affinity for 5-HT_{1A} receptors and their selectivity compared to other 5-HT or dopamine receptor subtypes (Table 5).

To quickly estimate the potential interest of such rigid compounds, a spiranic analogue (**8a**) of the 5-methoxy-3-(di-*n*-propylamino)-3,4-dihydro-2*H*-1-benzopyran (5-MeO-DPAC, **1**) was first synthesized. It appeared to be in the same range of affinity for 5-HT_{1A} receptor as **1** with a clearly higher selectivity over D_2 receptors.

After validation of the concept and after the results of molecular modeling, the spiropiperidine derivative **22** has been prepared. This compound showed a significant but rather lower affinity than the product **8a**. In order to reduce the number of syntheses necessary to improve both the affinity and the selectivity, we have first transposed to these spiro derivatives the results of the structure−activity relationships established in our previous works on 3-amino-3,4-dihydro-2*H*-1-benzopyrans

Table 5. IC_{50} (M) Values in the Binding Tests

compd	5-HT_{1A}	pred values for 5-HT_{1A}	5-HT_{1B}	5-HT_2	5-HT_3	D_1	D_2
1[a]	2.0×10^{-8}	3.7×10^{-8}	2.0×10^{-5}	6.0×10^{-5}		1.0×10^{-4}	5.0×10^{-8}
2[a]	2.0×10^{-10}	2.7×10^{-10}	5.0×10^{-6}	1.0×10^{-6}		4.0×10^{-5}	1.0×10^{-8}
(+)-**2**[a]	3.0×10^{-10}		1.0×10^{-5}	3.0×10^{-6}		4.0×10^{-5}	2.0×10^{-8}
(−)-**2**[a]	2.0×10^{-9}		6.0×10^{-6}	5.0×10^{-6}		2.0×10^{-5}	1.0×10^{-8}
5a	$(4.37 \pm 0.37) \times 10^{-6}$	1.2×10^{-6}	$>10^{-5}$	$>10^{-4}$			
6a[b]	$(2.98 \pm 0.20) \times 10^{-7}$	2.5×10^{-7}	4.40×10^{-6}	$(7.14 \pm 0.58) \times 10^{-5}$	$(1.02 \pm 0.22) \times 10^{-5}$		
7a	$(1.42 \pm 0.22) \times 10^{-5}$	3.3×10^{-5}	$>10^{-4}$	$(7.93 \pm 6.04) \times 10^{-4}$	$>10^{-4}$		
8a[b]	$(7.46 \pm 0.37) \times 10^{-8}$	1.1×10^{-8}	1.10×10^{-5}	$(1.12 \pm 0.11) \times 10^{-4}$	$(3.59 \pm 3.66) \times 10^{-6}$	$(1.20 \pm 0.43) \times 10^{-4}$	$(6.55 \pm 2.55) \times 10^{-6}$
8b[b]	$(1.30 \pm 0.22) \times 10^{-6}$	1.5×10^{-6}	$>10^{-4}$	$(2.33 \pm 0.31) \times 10^{-4}$	$(1.45 \pm 0.70) \times 10^{-5}$		
9[b]	$(3.85 \pm 0.42) \times 10^{-8}$	7.7×10^{-7}	$>10^{-5}$	$(3.64 \pm 2.06) \times 10^{-4}$	$(7.93 \pm 3.65) \times 10^{-6}$	$>10^{-4}$	$(7.14 \pm 5.01) \times 10^{-5}$
10[b]	$(1.01 \pm 0.10) \times 10^{-7}$	1.5×10^{-7}	2.70×10^{-6}	$(6.73 \pm 1.68) \times 10^{-5}$	$(3.97 \pm 3.47) \times 10^{-6}$		
11a[b]	$(1.06 \pm 0.04) \times 10^{-8}$	9.8×10^{-9}	3.80×10^{-6}	$(6.61 \pm 0.74) \times 10^{-6}$	$(3.07 \pm 0.64) \times 10^{-5}$	$(3.83 \pm 0.68) \times 10^{-5}$	$(9.27 \pm 2.03) \times 10^{-7}$
(+)-**11a**[b]	$(6.72 \pm 0.74) \times 10^{-9}$		$(1.30 \pm 0.42) \times 10^{-6}$	$(8.86 \pm 1.24) \times 10^{-7}$	$(1.21 \pm 0.12) \times 10^{-5}$	$(2.24 \pm 1.04) \times 10^{-5}$	$(1.31 \pm 0.17) \times 10^{-6}$
(−)-**11a**[b]	$(1.15 \pm 0.12) \times 10^{-6}$		$(3.11 \pm 0.57) \times 10^{-5}$	$(1.39 \pm 0.37) \times 10^{-5}$	$(1.06 \pm 0.17) \times 10^{-5}$	$(2.33 \pm 1.72) \times 10^{-5}$	$(1.13 \pm 0.44) \times 10^{-5}$
11b[b]	$(1.78 \pm 0.15) \times 10^{-7}$	7.3×10^{-8}	3.80×10^{-6}	$(5.95 \pm 3.27) \times 10^{-6}$	$(1.25 \pm 2.36) \times 10^{-5}$		
12	$(8.14 \pm 1.13) \times 10^{-5}$	1.7×10^{-5}	$(2.46 \pm 1.48) \times 10^{-4}$	$>10^{-4}$	$>10^{-4}$		
22	$(4.20 \pm 0.23) \times 10^{-7}$	1.8×10^{-7}	$(6.91 \pm 1.48) \times 10^{-5}$	$(4.14 \pm 0.59) \times 10^{-5}$	$(1.49 \pm 0.38) \times 10^{-5}$	1.0×10^{-4}	$(1.47 \pm 0.07) \times 10^{-5}$
23	$(8.81 \pm 0.23) \times 10^{-9}$	9.4×10^{-9}	$(2.21 \pm 0.46) \times 10^{-6}$	$(2.91 \pm 0.47) \times 10^{-6}$	$(4.14 \pm 0.54) \times 10^{-5}$	$(2.34 \pm 0.20) \times 10^{-5}$	$(2.84 \pm 0.95) \times 10^{-6}$
24	$(6.80 \pm 0.35) \times 10^{-8}$	9.1×10^{-8}	$(5.11 \pm 1.52) \times 10^{-5}$	$(2.11 \pm 0.28) \times 10^{-5}$	$(1.18 \pm 0.15) \times 10^{-4}$	$(3.80 \pm 0.63) \times 10^{-5}$	$(4.22 \pm 0.43) \times 10^{-6}$
25	$(6.28 \pm 0.38) \times 10^{-8}$	1.5×10^{-7}	$(1.12 \pm 0.54) \times 10^{-5}$	$(8.39 \pm 1.30) \times 10^{-6}$	$(6.65 \pm 0.83) \times 10^{-5}$	$(3.83 \pm 0.43) \times 10^{-5}$	$(4.30 \pm 1.02) \times 10^{-6}$
8-OH-DPAT[a]	2.4×10^{-9}			4.9×10^{-5}		$>10^{-4}$	8.0×10^{-7}
buspirone[a]	6.0×10^{-8}			1.0×10^{-5}		2.7×10^{-5}	4.0×10^{-6}
ipsapirone[a]	3.5×10^{-8}		5.0×10^{-5}	7.5×10^{-6}		1.2×10^{-5}	$>10^{-4}$

[a] These compounds are described in ref 31. [b] These compounds were tested as oxalate.

that lead us to the selection of (+)-**2**, *i.e.*: importance of the 5-methoxy group on the benzopyran moiety and best results obtained with a four-methylene unit alkyl spacer and a spiranic imide substituent (8-azaspiro[4.5]-decane-7,9-dione) on the basic nitrogen atom.

Compounds **11a** and **23**, analogues of **2,** were prepared in the spiropyrrolidine and spiropiperidine series, respectively. After preliminary evaluation, both compounds proved to be almost equivalent and potent ligands for the 5-HT$_{1A}$ binding sites with IC$_{50}$ values of respectively 1.06×10^{-8} and 8.81×10^{-9} M with a high selectivity compared to 5-HT$_{1B}$, 5-HT$_2$, D$_1$, and D$_2$ binding sites. Nevertheless **11a** and **23** were found to have a slightly lower affinity than **2**.

Some of the structural modulations already done in the 3-aminobenzopyran series were reproduced in order to confirm or partially invalidate the structure−activity relationships previously established. These are as follows: (a) 5-Methoxy- and 5-hydroxy-substituted compounds **8a**, **11a**, and **9** have clearly a higher affinity for 5-HT$_{1A}$ binding sites than their non-5-substituted analogues **8b** and **11b** (in the 3-aminobenzopyran series the three compounds were equipotent). (b) When a comparison is possible (**8a**, **9**), 5-methoxy and 5-hydroxy substituents are almost equivalent in terms of affinity for the 5-HT$_{1A}$ binding sites with however a slightly better selectivity for the 5-hydroxy-substituted compound **9**. (c) Replacement of the tertiary amine by a secondary one (**6a**, **8a**) results in a slight decrease in affinity for the 5-HT$_{1A}$ receptors. (d) Suppression of the basicity of the amine by acetylation (**12**) or its inclusion in a lactam by transformation of the spiropyrrolidine in a spiropyrrolidone (**5a**, **7a**) results in a dramatic decrease in affinity. (e) Sulfonamides or carboxamides **24** and **25** appear to have an affinity 10 times lower for the 5-HT$_{1A}$ binding sites than their spiroimide analogue **23**. By contrast, their selectivity remains equivalent. (f) Surprisingly, compound **10** with a 5-spiroimidoalkyloxy substituent retains some affinity for the 5-HT$_{1A}$ receptors. (g) With the exception of (+)-**11a** with an IC$_{50}$ of 8.86×10^{-7} M for 5-HT$_2$ binding sites and **11a** with an IC$_{50}$ of 9.27×10^{-7} M for D$_2$ binding sites, all the prepared compounds showed moderate affinity for non-5-HT$_{1A}$-tested binding sites (5-HT$_{1B}$, 5-HT$_2$, D$_1$, D$_2$) with IC$_{50}$ values ranging from 10^{-4} to 10^{-6} M. (h) It is impossible to come to a clear conclusion on the influence of the size of the spiranic cyclic amine. Spiropyrrolidine appears to be a slightly higher affinity ligand for the 5-HT$_{1A}$ receptors than the spiropiperidine analogues when the substituent is a propyl (**8a**, **22**). It is the opposite with a spiroimide substituent (**11a**, **23**). (i) Generally speaking, spiranic compounds appeared to be slightly lower affinity ligands for the 5-HT$_{1A}$ binding sites than their 3-amino-3,4-dihydro-2*H*-1-benzopyran analogues. (j) Compound **11a** was resolved in its two enantiomers. As in the 3-aminobenzopyran series, the dextrorotatory enantiomer (+)-**11a** shows a higher affinity for the 5-HT$_{1A}$ binding sites than its levorotatory analogue (−)-**11a**. It is probably due to the higher rigidity of the molecule's structure that the difference in affinity between the two enantiomers appears much higher than in the benzopyran series (compounds (+)-**2**, (−)-**2**).

In conclusion, this preliminary evaluation allowed us to obtain new compounds, among which the compounds (+)-**11a** and **23** have a greater affinity for 5-HT$_{1A}$ receptors than buspirone with an equivalent selectivity compared to other 5-HT or dopamine receptor subtypes.

Table 6. Effects of (±)-**11a** and Its Isomers on Forskolin-Activated Adenylate Cyclase in Rat Hippocampi

compd	concentration (M)	[^{32}P]cAMP (%)[a]
(+)-**11a**	0	100 ± 2
	10^{-10}	97 ± 1
	10^{-9}	91 ± 2
	10^{-8}	88 ± 3* [b]
	10^{-7}	84 ± 2*
	10^{-6}	82 ± 2*
(−)-**11a**	10^{-8}	98 ± 1
	10^{-7}	92 ± 2
	10^{-6}	86 ± 2*
	10^{-5}	81 ± 2*
(±)-**11a**	10^{-7}	80 ± 2*

[a] Each value is the mean ± standard error of three determinations in two independent experiments. [b] An asterisk indicates $p < 0.05$ (versus 100%) using student's *t*-test.

Pharmacology

Due to its high affinity for 5-HT$_{1A}$ binding sites and its relative selectivity regarding other 5-HT receptor subtypes (5-HT$_{1B}$, 5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_3$) and D$_1$ and D$_2$ dopaminergic receptors, compound **11a** was selected for further investigation among the above-mentioned compounds. Compound **11a** was thus tested *in vitro* to determine the nature of its interaction on postsynaptic 5-HT$_{1A}$ receptors: *i.e.*, the effects on forskolin-induced cAMP in rat hippocampus (the inhibition of which is believed to be mediated through 5-HT$_{1A}$ receptors[55]). For one concentration, the potency of its effect was compared with that of its enantiomers, compounds (+)-**11a** and (−)-**11a**. The isomer with the highest affinity for the 5-HT$_{1A}$ receptors was then evaluated *in vivo* in animal tests predictive of (i) antidepressant-like effects, using the forced swim test in mice and rats and the tail suspension test in mice, and (ii) anxiolytic-like activity using the light−dark box test and the elevated plus maze in mice.

Results. As indicated in Table 5, the dextrorotary enantiomer of compound **11a** showed the highest affinity for the 5-HT$_{1A}$ binding sites as well as the highest selectivity with regards to D$_2$ dopaminergic receptor subtypes. In comparison, the levorotatory enantiomer had moderate affinity for 5-HT$_{1A}$ receptors. Table 6 shows the effects of both enantiomers of compound **11a** on adenylate cyclase activity in rat hippocampus.

The results indicate a clear concentration-dependent inhibition of cyclase activity by both enantiomers with a maximum of 20%. One possible explanation for this equipotency of both enantiomers could be that one enantiomer is a partial agonist for the 5-HT$_{1A}$ receptors and the other a full agonist. By comparison, the maximum inhibition of the forskolin-stimulated cAMP production by 8-OH-DPAT seems to be around 25%.[56]

To confirm the involvement of 5-HT$_{1A}$ receptors in this effect, both enantiomers (+)-**11a** and (−)-**11a** were tested in the presence of tertatolol, a 5-HT$_{1A}$ receptor antagonist (10^{-6} M). Table 7 shows that tertatolol completely antagonized the inhibition of cAMP induced by (+)-**11a** and (−)-**11a**, which is consistent with an effect occurring *via* 5-HT$_{1A}$ receptor stimulation.

Compound (+)-**11a** was tested *in vivo* in different tests predictive of psychotropic activity: (1) In the forced

Table 7. Effects of (+)-**11a** and (−)-**11a** on Forskolin-Activated Adenylate Cyclase in Rat Hippocampi in the Absence and Presence of 1 μM (−)-Tertatolol

addition	[^{32}P]cAMP (%)[a]
vehicle	100 ± 3
(−)-tertatolol (1 μM)	103 ± 2
(+)-**11a** (1 μM)	79 ± 3* [b]
(−)-**11a** (1 μM)	86 ± 2*
(−)-tertatolol	98 ± 2
(+)-**11a**	98 ± 2
(−)-tertatolol	101 ± 2
(−)-**11a**	101 ± 2

[a] Each value is the mean ± standard error of three determinations in two experiments. [b] An asterisk indicates $p < 0.05$ (versus 100%) using student's *t*-test.

swim test, predictive of an antidepressant-like activity, compound (+)-**11a** decreased the duration of the immobility in both mice and rats; the minimum significative doses (MSAD) were 32 mg/kg ip in mice and 16 mg/kg ip in rats. In comparison the MSAD for imipramine was 32 mg/kg ip. (2) In the tail suspension test, derived from the latter test, (+)-**11a** tended to decrease the duration of immobility from the dose of 32 mg/kg ip and significantly increased the power of the movements, an effect shown by several antidepressants, including desipramine with a MSAD of 32 mg/kg ip. Compound (+)-**11a** showed a potent antidepressant activity in both mice and rats in different paradigms. (3) In the light−dark box paradigm, predictive of an anxiolytic-like activity in mice, (+)-**11a** increased the time spent in the light box as well as the number of transitions with a MSAD of 7.5 mg/kg ip. In the same conditions, the MSAD for buspirone was 10 mg/kg ip. (4) In the elevated plus maze, also predictive of an anxiolytic-like activity, (+)-**11a** did not increase the number of entries into the open arms nor the time spent in these arms. However, (+)-**11a** significantly increased the number of entries into the closed arms (MSAD = 32 mg/kg ip) showing the lack of sedative effects. The reference compound clobazam increased both the number of entries in the open arms and the time spent with a MSAD of 16 mg/kg ip.

Conclusion

The results we have obtained within this spiranic series are in agreement with the structure−activity relationships previously established in our previous work on 3-amino-3,4-dihydro-2*H*-1-benzopyran derivatives and allowed us to prepare 5-HT$_{1A}$ ligands of high affinity and selectivity, among others, the spiroimido derivatives **11a** and **23**.

Quantitative structure−activity relationship analysis was evaluated according to the similarity indices approach. We have obtained good statistical results using PLS analysis of electrostatic fields and electrostatic potential similarity indices. The predictive power of this model has been shown by a good correlation between the calculated values of binding and the experimental data.

After resolution, the dextrorotatory enantiomer (+)-**11a** proved to be the most interesting according to the binding studies. (+)-**11a** proved to be active in compportmental tests predictive of psychotropic activities and is currently under preclinical investigation.

Experimental Section

Chemistry. General Comments. Melting points (uncorrected) were determined on a Kofler hot-stage apparatus. Infrared spectra were determined with a Perkin-Elmer 297 spectrometer. The proton NMR spectra were obtained on a Bruker AM 300 spectrometer (300 MHz). Chemical shifts are reported in parts per million (δ, ppm) downfield from tetramethylsilane (TMS) which was used as an internal standard. The deuterated NMR solvents containing 99.8% deuterium with 1% (v/v) TMS were obtained from Aldrich-Chimie. ^{1}H NMR coupling constants (*J* values) are listed in hertz (Hz), and spin multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Chemical ionization mass spectral data (MS) were reported on a R10-10C Nermag (70 eV) apparatus using chemical ionization (CI/NH_3) or electronic impact (EI) methods. Organic solvents were purified when necessary by the methods described by D. D. Perrin, W. L. F. Armarego, and D. R. Perrin *(Purification of Laboratory Chemicals*; Pergamon: Oxford, 1986) or were purchased from Aldrich or Janssen Chimica. All solutions were dried over anhydrous magnesium sulfate and evaporated on a Büchi rotatory evaporator. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel, 60 F_{254}), and spots were visualized with UV light or alcohol solution of ammonium cerium(IV) nitrate. Column chromatography was performed with Kieselgel 60 (70−230 mesh) silica gel (Merck) for gravity columns and Kieselgel 60 (230−400 mesh) silica gel (Merck) for flash columns. Where analyses in the tables are indicated by symbols of the elements, analytical results were within 0.4% of the theoretical values. All nonaqueous reactions were perfomed in oven-dried glassware under an atmosphere of argon. The column chromatography solvents employed were glass distilled, and solvent mixtures are reported as volume to volume ratios. Compounds **13**−**17** were a mixture of two diastereoisomeres which were not separated.

Methyl 3-(5-Methoxy-3-nitro-3,4-dihydro-2*H*-1-benzopyran-3-yl)propionate (4a). A magnetically stirred solution of **3a**[31,34] (4.000 g, 19.14 mmol) and benzyltrimethylammonium methoxide (0.4 mL) in methanol (MeOH) (60 mL) was warmed to 70 °C for 1.5 h, and the reaction mixture was then cooled to room temperature. The solvent was evaporated to dryness, and the product was extracted, after aqueous hydrolysis, with dichloromethane (CH_2Cl_2). The organic layer was dried ($MgSO_4$) and evaporated. The resulting oil was then purified by chromatography on a silica gel column (eluent: CH_2Cl_2/petroleum ether, 1/1) to give the expected compound **4a** (5.100 g) as an oil in 90% yield: IR (film) ν 1730 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.15−2.59 (m, 252H, 25**CH$_2$**), 2.91 (d, 1H, **CH$_2$**-Ar, *J* = 17.5), 3.57 (d, 1H, **CH$_2$**Ar, *J* = 17.5), 3.68 (s, 3H, COO**CH$_3$**), 3.84 (s, 3H, O**CH$_3$**), 4.10 (d, 1H, **CH$_2$**O, *J* = 11.6), 4.57 (d, 1H, **CH$_2$**O, *J* = 11.6), 6.48 (d, 1H, **H$_{arom}$**, *J* = 8.3), 6.51 (d, 1H, **H$_{arom}$**, *J* = 8.3), 7.11 (t, 1H, **H$_{arom}$**, *J* = 8.3). Anal. ($C_{14}H_{17}NO_6$) C, H, N.

Methyl 3 (3-Nitro-3,4-dihydro-2*H*-1-benzopyran-3-yl)-proprionate (4b). The titled compound **4b** (0.250 g) was prepared from **3**[31,34] (0.200 g, 1.12 mmol) in a manner analogous to the above procedure. The expected product **4b** was obtained in 85% yield as an oil: IR (film) ν 1730 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.20−2.50 (m, 252H, 25**CH$_2$**), 3.14 (d, 1H, **CH$_2$**Ar, *J* = 16.9), 3.67 (d, 1H, **CH$_2$**Ar, *J* = 16.9), 3.71 (s, 3H, **CH$_3$**O), 4.21 (d, 1H, **CH$_2$**O, *J* = 11.4), 4.64 (d, 1H, **CH$_2$**O, *J* = 11.4), 6.84−7.21 (m, 4H, **H$_{arom}$**). Anal. ($C_{13}H_{15}NO_5$) C, H, N.

5-Oxo-5′-methoxy-3′,4′-dihydrospiro[pyrrolidine-2,3′-(2′*H*)-benzopyran] (5a). The compound **4a** (5.100 g, 17.27 mmol) was dissolved in methanol (MeOH) (100 mL). Raney nickel (0.700 g, moist) was added, and the mixture was hydrogenated at atmospheric pressure at 60 °C overnight. The catalyst was removed by filtration, and the mixture was refluxed for 4 h. After cooling, the solvent was evaporated *in vacuo* to yield a solid which was purified by flash chromatography (eluent: CH_2Cl_2/MeOH, 8/2) to afford the derivative **5a** (3.660 g) as a solid in 91% yield: mp 210−211 °C; IR (KBr) ν 3250 (NH), 1670 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.94−2.16

(m, 2H, **CH$_2$**), 2.43−2.59 (m, 2H, **CH$_2$**CO), 2.75 (d, 1H, **CH$_2$**-Ar, J = 16.5), 2.87 (d, 1H, **CH$_2$**Ar, J = 16.5), 3.83 (s, 3H, **CH$_3$**O), 3.89 (d, 1H, **CH$_2$**O, J = 11.0), 3.94 (d, 1H, **CH$_2$**O, J = 11.0), 5.83 (br s, 1H, **NH**CO), 6.46 (d, 1H, **H$_{arom}$**, J = 8.3), 6.53 (d, 1H, **H$_{arom}$**, J = 8.3), 7.11 (t, 1H, **H$_{arom}$**, J = 8.3); MS (EI) m/z 233 (M^+). Anal. ($C_{13}H_{15}NO_3$) C, H, N.

5-Oxo-3′,4′-dihydrospiro[pyrrolidine-2,3′(2′*H*)-benzopyran] (5b). The compound **5b** was synthesized from the corresponding nitro ester **4b** (0.200 g, 0.75 mmol) according to the procedure described above. The pure product **5b** (0.150 g) was obtained in 96% yield as a solid: mp 179−180 °C; IR (KBr) ν 3225 (NH), 1675 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.91−2.17 (m, 2H, **CH$_2$**), 2.51 (t, 2H, **CH$_2$**CO, J = 8.2), 2.88 (d, 1H, **CH$_2$**Ar, J = 16.4), 2.97 (d, 1H, **CH$_2$**Ar, J = 16.4), 3.94 (d, 1H, **CH$_2$**O, J = 10.3) 3.98 (d, 1H, **CH$_2$**O, J = 10.3), 6.04 (br s, 1H, **NH**CO), 6.83−7.18 (m, 4H, **H$_{arom}$**). Anal. ($C_{12}H_{13}NO_2$) C, H, N.

1-*n*-Propyl-5-oxo-5′-methoxy-3′,4′-dihydrospiro[pyrrolidine-2,3′(2′*H*)-benzopyran] (7a). To a suspension of sodium hydride (NaH) (60% dispersion in mineral oil, 0.120 g, 4.70 mmol) in *N*,*N*-dimethylformamide (DMF) (18 mL) was added a solution of **5a** (1.000 g, 4.29 mmol) in DMF (2 mL). The mixture was stirred at 60 °C for 1 h, and then 1-iodopropane (3.610 g, 21.30 mmol) was added. Stirring was continued for 8 h, and the reaction mixture was cooled to room temperature. The solvent was removed *in vacuo* and the residue taken up in CH_2Cl_2 (20 mL). The organic layer was washed with water, dried ($MgSO_4$), and concentrated. The residue was chromatographed over silica gel (eluent: Et_2O/CH_2Cl_2, 1/1) to give the expected product **7a** (0.830 g) as a solid in 64% yield: mp 89−90 °C; IR (KBr) ν 1670 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, **CH$_3$**CH$_2$, J = 8.5), 1.50−1.66 (m, 2H, **CH$_2$**CH$_3$), 1.74−1.84 (m, 1H, **CH$_2$**), 2.07−2.16 (m, 1H, **CH$_2$**), 2.44 (t, 2H, **CH$_2$**-CO, J = 8.1), 2.69 (dd, 1H, **CH$_2$**Ar, J = 17.1, 2.4), 2.90 (d, 1H, **CH$_2$**Ar, J = 17.1), 3.00−3.23 (m, 2H, **CH$_2$**N), 3.84 (s, 3H, **CH$_3$**O), 3.88 (dd, 1H, **CH$_2$**O, J = 10.3, 2.4), 3.97 (d, 1H, **CH$_2$**O, J = 10.3), 6.48 (d, 1H, **H$_{arom}$**, J = 8.3), 6.52 (d, 1H, **H$_{arom}$**, J = 8.3), 7.11 (t, 1H, **H$_{arom}$**, J = 8.3); MS (EI) m/z 275 (M^+). Anal. ($C_{16}H_{21}NO_3$) C, H, N.

5′-Methoxy-3′,4′-dihydrospiro[pyrrolidine-2,3′(2′*H*)-benzopyran] (6a). To a stirring solution of **5a** (0.100 g, 0.42 mmol) in dry tetrahydrofuran (THF) (5 mL) was added dropwise borane−methyl sulfide complex (2 M solution in tetrahydrofuran, 0.85 mL). The solution was heated under reflux for 4 h, and then the solvent was evaporated to dryness. The residue was heated on a steam bath for 1.5 h with 2 M HCl (16 mL) and MeOH (8 mL). The solution was cooled and basified with 2 M NaOH, and the product was extracted with CH_2Cl_2. The combined extracts were washed with water, dried ($MgSO_4$), and evaporated to dryness. The residue was purified by column chromatography (eluent: CH_2Cl_2/MeOH, 95/5) to give the desired product **6a** (0.070 g) as an oil in 76% yield: IR (film) ν 3350 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.56−1.95 (m, 252H, 25**CH$_2$**), 2.21 (br s, 1H, **NH**), 2.68 (s, 2H, **CH$_2$**Ar), 2.96−3.17 (m, 2H, **CH$_2$**N), 3.80 (s, 3H, **CH$_3$**O), 3.82 (s, 2H, **CH$_2$**O), 6.43 (d, 1H, **H$_{arom}$**, J = 8.3), 6.52 (d, 1H, **H$_{arom}$**, J = 8.3), 7.06 (t, 1H, **H$_{arom}$**, J = 8.3); MS (EI) m/z 219 (M^+). Anal. ($C_{13}H_{17}NO_2$) C, H, N.

3′,4′-Dihydrospiro[pyrrolidine-2,3′(2′*H*)-benzopyran] (6b). From compound **5b** (1.220 g, 6.00 mmol), the compound **6b** was prepared according to the procedure described above. The expected amine **6b** was obtained in 72% yield as an oil: IR (film) ν 3350 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.55−1.96 (m, 252H, 25**CH$_2$**), 2.18 (br s, 1H, **NH**), 2.78 (d, 1H, **CH$_2$**Ar, J = 16.6), 2.84 (d, 1H, **CH$_2$**Ar, J = 16.6), 2.94−3.14 (m, 2H, **CH$_2$**N), 3.87 (s, 2H, **CH$_2$**O), 6.83−7.15 (m, 4H, **H$_{arom}$**); MS (EI) m/z (M^+). Anal. ($C_{12}H_{15}NO$) C, H, N.

1-*n*-Propyl-5′-methoxy-3′,4′-dihydrospiro[pyrrolidine-2,3′(2′*H*)-benzopyran] (8a). Method A. A mixture of **6a** (0.100 g, 0.46 mmol), 1-iodopropane (0.230 g, 1.37 mmol), and potassium carbonate (0.190 g, 1.37 mmol) in DMF (5 mL) was warmed with stirring at 60 °C for 3 h. The reaction mixture was cooled to room temperature, and the solvent was removed under reduced pressure. After hydrolysis the product was extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$) and evaporated to dryness. Purification by column chromatography of the resulting crude (eluent: Et_2O/petroleum ether, 2/8) gave the compound **8a (**0.100 g) as an oil in 84% yield: IR (film) ν 2960−2800 (CH_2, CH_3) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3H, **CH$_3$**CH$_2$, J = 7.4), 1.42−1.97 (m, 6H, **CH$_2$**CH$_3$, 25**CH$_2$**), 2.38−2.61 (m, 3H, **CH$_2$**CH$_2$CH$_3$, **CH$_2$**Ar), 2.70 (d, 1H, **CH$_2$**Ar, J = 17.0), 2.82−3.03 (m, 2H, **CH$_2$**N), 3.78 (d, 1H, **CH$_2$**O, J = 10.3), 3.81 (s, 3H, **CH$_3$**O), 3.83 (d, 1H, **CH$_2$**O, J = 10.3), 6.42 (d, 1H, **H$_{arom}$**, J = 8.3), 6.48 (d, 1H, **H$_{arom}$**, J = 8.3), 7.06 (t, 1H, **H$_{arom}$**, J = 8.3); MS (EI) m/z 261 (M^+). Anal. ($C_{16}H_{23}NO_2$) C, H, N.

Method B. To a mixture of **7a** (0.150 g, 0.54 mmol) in dry THF (7 mL) was added dropwise borane−methyl sulfide complex (2 M solution in tetrahydrofuran, 0.55 mL). The solution was heated under reflux overnight, and the solvent was evaporated to dryness. The residue was heated on a steam bath for 1.5 h with 2 M HCl (12 mL) and methanol (6 mL). The solution was cooled and basified with 2 M NaOH. The product was extracted with CH_2Cl_2 and dried ($MgSO_4$), and the solvent were evaporated to dryness. The residue was purified by column chromatography (eluent: CH_2Cl_2/MeOH) to afford in 81% yield the desired product **8a** (0.115 g) which was identical with that obtained from method A.

1-*n*-Propyl-3′,4′-dihydrospiro[pyrrolidine-2,3′(2′*H*)-benzopyran] (8b). The compound **8b** was prepared from the corresponding secondary amine (1.300 g, 6.87 mmol) according to method A, in the presence of 1-iodopropane (3.460 g, 20.6 mmol). The pure product **8b** (0.970g) was obtained as an oil in 61% yield: IR (film) ν 2980−2800 (CH_2, CH_3) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3H, **CH$_3$**CH$_2$, J = 7.4), 1.43−2.01 (m, 6H, **CH$_2$**CH$_3$, 25**CH$_2$**), 2.39−2.59 (m, 3H, **CH$_2$**CH$_2$CH$_3$, **CH$_2$**Ar), 2.83−2.96 (m, 2H, **CH$_2$**N), 3.02 (d, 1H, **CH$_2$**Ar, J = 16.1), 3.83 (dd, 1H, **CH$_2$**O, J = 10.3, 2.4), 3.88 (dd, 1H, **CH$_2$**O, J = 10.3), 6.78−7.11 (m, 4H, **H$_{ar}$**); MS (EI) m/z 231 (M^+). Anal. ($C_{15}H_{21}NO$) C, H, N.

1-*n*-Propyl-5′-hydroxy-3′,4′-dihydrospiro[pyrrolidine-2,3′(2′*H*)-benzopyran] (9). To a solution of **8a** (1.100 g, 4.20 mmol) in acetic acid (22 mL) was added hydrobromic acid (48%) (11 mL). The mixture was refluxed at 130−140 °C for 5 h and cooled, and the solvent was evaporated to dryness. The material was poured with agitation into saturated sodium bicarbonate (40 mL). The product was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The crude was purified by silica gel column chromatography (eluent: Et_2O/petroleum ether, 1/1) to give the product **9** (0.910 g) as a solid in 88% yield: mp 185−186 °C; IR (KBr) ν 3450−3250 (OH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.83 (t, 3H, **CH$_3$**CH$_2$, J = 7.4), 1.30−1.45 (m, 3H, **CH$_2$**CH$_3$, **CH$_2$**), 1.65−1.80 (m, 3H, **CH$_2$**), 2.32 (d, 1H, **CH$_2$**Ar, J = 17.0), 2.46−2.50 (m, 2H, **CH$_2$**-CH$_2$CH$_3$), 2.59 (d, 1H, **CH$_2$**Ar, J = 17.0), 2.71−2.90 (m, 2H, **CH$_2$**N), 3.69 (d, 1H, **CH$_2$**O, J = 10.3), 3.76 (d, 1H, **CH$_2$**O, J = 10.3), 6.21 (d, 1H, **H$_{arom}$**, J = 8.3), 6.32 (d, 1H, **H$_{arom}$**, J = 8.3), 6.81 (t, 1H, **H$_{arom}$**, J = 8.3), 9.32 (br s, 1H, **OH**); MS (EI) m/z 247 (M^+). Anal. ($C_{15}H_{21}NO_2$), C, H, N.

1-*n*-Propyl-5′-[[4′-(7′,9′-dioxo-8′-azaspiro[4′.5′]decanyl)-butyl]oxy]-3′,4′-dihydrospiro[pyrrolidine-2,3′(2′*H*)-benzopyran] (10). A mixture of **9** (0.058 g, 0.23 mmol), 8-(4-bromobutyl)-8-azaspiro[4.5]decane-7,9-dione (0.076 g, 0.25 mmol), potassium carbonate (0.095 g, 0.069 mmol), and a catalytic amount of potassium iodide in DMF (3 mL) was warmed with stirring at 60 °C for 12 h. The reaction mixture was cooled to room temperature, and the solvent was removed under reduced pressure. After hydrolysis, the product was extracted with CH_2Cl_2 from the resulting crude. The organic solvent was dried ($MgSO_4$) and evaporated to dryness. The residual oil was purified by flash chromatography (eluent: Et_2O/CH_2Cl_2, 1/1) to give the expected derivative **10** (0.085 g) as an oil in 79% yield: IR (film) ν 1715, 1660 (C=O) cm^{-1}; ^{1}H NMR (CDC1$_3$) δ 0.94 (t, 3H, **CH$_3$**CH$_2$, J = 7.4), 1.48−2.03 (m, 18H, **CH$_2$**), 2.40−2.68 (m, 7H, **CH$_2$**CO, **CH$_2$**Ar, **CH$_2$**CH$_3$), 2.74 (d, 1H, **CH$_2$**Ar, J = 17.0), 2.90−3.06 (m, 2H, **CH$_2$**N), 3.82−3.92 (m, 4H, **CH$_2$**O, **CH$_2$**NCO), 3.95−4.04 (m, 2H, **CH$_2$**O), 6.41 (d, 1H, **H$_{arom}$**, J = 8.3), 6.48 (d, 1H, **H$_{arom}$**, J = 8.3), 7.04 (t, 1H, **H$_{arom}$**, J = 8.3); MS (EI) m/z 468 (M^+). Anal. ($C_{28}H_{40}N_2O_4$) C, H, N.

1-[4-(7,9-Dioxo-8-azaspiro[4.5]decanyl)butyl]-5′-methoxy-3′,4′-dihydrospiro[pyrrolidine-2,3′(2′*H*)-benzopyran] (11a). A mixture of **6a** (1.200 g, 5.47 mmol), 8-(4-bromobutyl)-8-azaspiro[4.5]decane-7,9-dione (1.800 g, 6.0 mmol), triethylamine (1.660 g, 16.40 mmol), and a catalytic amount of potassium iodide in DMF (15 mL) was warmed with stirring at 60 °C overnight. The reaction mixture was cooled to room temperature, and the solvent was removed under reduced pressure. After hydrolysis, the product was extracted with CH_2Cl_2. The solvent was dried ($MgSO_4$) and evaporated to dryness. Purification by column chromatography of the resulting crude (eluent: Et_2O/CH_2Cl_2, 1/2) was performed to give the desired product **11a** (1.300 g) as an oil in 54% yield: IR (film) ν 1720, 1665 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35−1.95 (m, 16H, **CH_2**), 2.40−2.56 (m, 3H, **CH_2**N, **CH_2**Ar), 2.57 (s, 4H, **CH_2**CO), 2.66 (d, 1H, **CH_2**Ar, J = 16.8), 2.76−3.03 (m, 2H, **CH_2**N), 3.69−3.79 (m, 4H, **CH_2**O, **CH_2**NCO), 3.81 (s, 3H, **CH_3**O), 6.41 (d, 1H, **H_{arom}**, J = 8.3), 6.46 (d, 1H, **H_{arom}**, J = 8.3), 7.04 (t, 1H, **H_{ar}**, J = 8.3); MS (EI) m/z 440 (M^+). Anal. ($C_{26}H_{36}N_2O_4$) C, H, N.

1-[4-(7,9-Dioxo-8-azaspiro[4.5]decanyl)butyl]-3′,4′-dihydrospiro[pyrrolidine-2,3′(2′*H*)-benzopyran] (11b). The compound **11b** was prepared from the corresponding secondary amine **6b** (1.500 g, 7.90 mmol) according to the procedure described above in the presence of 8-(4-bromobutyl)-8-azaspiro-[4.5]decane-7,9-dione (2.600 g, 8.70 mmol) and triethylamine (2.400 g, 23.7 mmol). The expected product **11b** (1.800 g) was obtained as an oil in 55% yield: IR (film) ν 1715, 1660 (C=O) cm^{-1}; ^1H NMR ($CDC1_3$) δ 1.40−2.00 (m, 16H, **CH_2**), 2.44 (d, 1H, **CH_2**Ar, J = 16.1), 2.48−2.56 (m, 2H, **CH_2**N), 2.58 (s, 4H, **CH_2**CO), 2.79−2.96 (m, 2H, **CH_2**N), 3.00 (d, 1H, **CH_2**Ar, J = 16.1), 3.73−3.91 (m, 4H, **CH_2**O, **CH_2**NCO), 6.77−7.10 (m, 4H, **H_{arom}**); MS (EI) m/z 410 (M^+). Anal. ($C_{25}H_{34}N_2O_3$) C, H, N.

(+)- and (−)-5′-Methoxy-3′,4′-dihydrospiro[pyrrolidine-2,3′(2′*H*)-benzopyran] ((+)-6a) and (−)-6a). **6a** (4.500 g, 20.50 mmol) was dissolved in MeOH (20 mL). (−)-1,1′-Binaphthyl-2,2′-diyl hydrogen phosphate (BNP) (5.000 g, 14.30 mmol), dissolved in a mixture of CH_2Cl_2/MeOH (1/9) (240 mL), was added to the previous solution. After stirring for 3 h, the solvent was removed under reduced pressure. This resulting residue was recrystallized once from acetonitrile and then three times from ethanol. The obtained salt was suspended in water and basified with aqueous ammonia. The free base so obtained was extracted with AcOEt to dryness, affording the crude optically enriched free base. The remaining traces of BNP, NH_4 salts were removed by flash chromatography over neutralized silica gel (eluent: AcOEt) yielding the pure free base (−)-**6a** (1.190 g) as an oil in 53% yield: $[\alpha]^{20}_D$ = −18° ($CHC1_3$, *c* 0.76). By following essentially the same procedure but substituting (+)-BNP for (−)-BNP, the remaining enantiomer was obtained as an oil, (+)-**6a**: $[\alpha]^{20}_D$ = +18 °C ($CHC1_3$, *c* 0.85). The optical purity was determined by HPLC (Chiracel OJ column, 0.8 mL/min, hexane/*i*-PrOH, 99/1). The purity of the two enantiomers was better than 99%.

(+)- and (−)-1-[4-(7,9-Dioxo-8-azaspiro[4.5]decanyl)-butyl]-5′-methoxy-3′,4′-dihydrospiro[pyrrolidine-2,3′(2′*H*)-benzopyran] ((+)-11a) and (−)-11a). According to the same method as described for the preparation of the racemic compound **11a**, the expected products (+)-**11a** and (−)-**11a** were obtained in 53% and 43% yields, respectively. (+)-**11a**: $[\alpha]^{20}_D$ = +15° ($CHCl_3$, *c* 0.65). (−)-**11a**: $[\alpha]^{20}_D$ = −16° ($CHCl_3$, *c* 0.71).

1-Acetamido-5′-methoxy-3′,4′-dihydrospiro[pyrrolidine-2,3′(2′*H*)-benzopyran] (12). A solution of **6a** (0.500 g, 2.28 mmol) in CH_2Cl_2 (10 mL) containing triethylamine (0.250 g, 2.50 mmol) was treated with acetic anhydride (0.256 g, 2.50 mmol). The mixture was stirred at room temperature for 30 min, and the CH_2Cl_2 solution was evaporated to dryness. To the resulting crude was added water (10 mL), and the product was extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$), and solvent was evaporated to dryness. The crude was purified by column chromatography (eluent: AcOEt) to afford the expected product **12** (0.570 g) as a solid in 96% yield: mp 120−121 °C; IR (KBr) ν 1640 (C=O) cm^{-1}; ^1H NMR ($CDC1_3$) δ 1.78−2.04 (m, 252H, 25**CH_2**), 2.06 (s, 3H, **CH_3**CO), 2.52 (dd, 1H, **CH_2**Ar, J = 16.6, 2.6), 3.50−3.63 (m, 2H, **CH_2**-NCO), 3.67 (d, 1H,**CH_2**Ar, J = 16.6), 3.76 (dd, 1H, **CH_2**O, J = 10.2, 2.6), 3.80 (s, 3H, **CH_3**O), 4.96 (d, 1H, **CH_2**O, J = 10.2) 6.42 (d, 1H, **H_{arom}**, J = 8.3), 6.50 (d, 1H, **H_{arom}**, J = 8.3), 7.06 (t, 1H, **H_{arom}**, J = 8.3); MS (EI) m/z 261 (M^+). Anal. ($C_{15}H_{19}NO_3$) C, H, N.

Methyl 4-Hydroxy-4-(5-methoxy-3-nitro-3,4-dihydro-2*H*-1-benzopyran-3-yl)butanoate (13). 5-Methoxy-3-nitro-2*H*-1-benzopyran (**3a**)[31,34] (1.500 g, 7.17 mmol) was dissolved in dry tetrahydrofuran (THF) (10 mL), and *n*-butyllithium (5.4 mL of 1.6 M solution in hexane, 8.60 mmol) was added dropwise at −78 °C. The mixture was stirred at −78 °C for 1 h. *tert*-Butyldimethylsilyl chloride (1.300 g, 8.60 mmol) in solution in THF (8 mL) was added slowly at −78 °C. After stirring for 1 h at −78 °C, *n*-tetrabutylammonium fluoride (2.700 g, 8.60 mmol) in THF (10 mL) and methyl 4-oxobutanoate (1.000 g, 8.60 mmol) in THF (6 mL) were added dropwise at −78 °C. The reaction mixture was stirred for 1 h at −78 °C and then allowed to reach room temperature for 48 h and hydrolyzed with water. The crude product was extracted with CH_2Cl_2. The organic layer was dried, and the solvents were removed under reduced pressure. The expected compound **13** was obtained after column chromatography (eluent: CH_2Cl_2/MeOH, 99/1) as an oil (1.420 g) in 61% yield: IR (film) ν 3600−3200 (OH), 1720 (C=O), 1235 (C−O−C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.56−1.82 (m, 1H, **CH_2**), 1.85−2.14 (m, 1H, **CH_2**), 2.46−2.66 (m, 2H, **CH_2**$COOCH_3$), 2.85 and 3.22 (2d, 1H, **CH_2**Ar, J = 18.0), 3.52 (br s, 1H, **OH**), 3.52 and 3.65 (2dd, 1H, Ar**CH_2**, J = 18.0, 2.5), 3.70 and 3.72 (2s, 3H, COO**CH_3**), 3.86 (s, 3H, O**CH_3**), 3.92 and 4.17 (2dd, 1H, **CH**OH, J = 10.7, 2.3), 4.14 and 4.32 (2d, 1H, **CH_2**O, J = 11.8), 4.70 and 4.89 (2dd, 1H, **CH_2**O, J = 11.8, 2.5), 6.48 (d, 1H, **H_{arom}**, J = 8.3), 6.51 (d, 1H, **H_{arom}**, J = 8.3), 7.08 (t, 1H, **H_{arom}**, J = 8.3). Anal. ($C_{15}H_{19}NO_7$) C, H, N.

Methyl 4-(Methoxymethoxy)-4-(5-methoxy-3-nitro-3,4-dihydro-2*H*-1-benzopyran-3-yl)butanoate (14). Alcohol **13** (1.350 g, 4.15 mmol) in dry chloroform ($CHCl_3$) (10 mL), diisopropylethylamine (3.220 g, 24.92 mmol), and chloromethyl methyl ether (1.86 mL, 24.92 mmol) were refluxed for 5 h. The mixture was allowed to cool to room temperature and hydrolyzed, and the product was extracted with CH_2Cl_2. After column chromatography (eluent: CH_2Cl_2/MeOH, 95/5), the protected alcohol **14** was given as an oil (1.000 g) in 65% yield: IR (film) ν 1720 (C=O), 1235 (C−O−C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.76−2.03 (m, 2H, **CH_2**), 2.43−2.57 (m, 2H, **CH_2**-$COOCH_3$), 2.98 and 3.13 (2d, 1H, **CH_2**Ar, J = 18.3), 3.37 and 3.39 (2s, 3H, **CH_3**OCH_2), 3.57 and 3.68 (2dd, 1H, **CH_2**Ar, J = 18.3, 2.7), 3.68 (s, 3H, COO**CH_3**), 3.85 (s, 3H, O**CH_3**), 4.08 and 4.19 (2d, 1H, **CH_2**O, J = 11.5), 4.13−4.19 (m, 1H, **CH**), 4.57−4.67 (m, 2H, O**CH_2**OCH_3), 4.72 and 4.76 (td, 1H, **CH_2**O, J = 11.5, 2.7), 6.49 (d, 2H, **H_{arom}**, J = 8.2), 7.08 (t, 1H, **H_{arom}**, J = 8.2). Anal. ($C_{17}H_{22}NO_8$) C, H, N.

3-(Methoxymethoxy)-6-oxo-5′-methoxy-3′,4′-dihydrospiro[piperidine-2,3′(2′*H*)-benzopyran] (15). Methyl ester **14** (1.600 g, 4.34 mmol) was dissolved in MeOH (12 mL). Raney nickel (0.640 g) was added, and the mixture was hydrogenated under hydrogen pressure (45 psi), in a Parr apparatus, at 45 °C. After 24 h, catalyst nickel was removed by filtration and washed with MeOH. After evaporation of the solvent, the crude product was chromatographed (eluent: CH_2Cl_2/MeOH, 95/5) to afford the expected amide **15** as an oil (1.070 g) in 80% yield: IR (film) ν 3160 (NH), 1695 (C=O), 1230 (C−O−C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.05−2.22 (m, 2H, **CH_2**CH), 2.35−2.64 (m, 2H, **CH_2**CO), 2.51 and 2.70 (dd, d, 1H, Ar**CH_2**, J = 17.2, 2.2), 3.13 and 3.38 (2d, 1H, Ar**CH_2**, J = 17.2), 3.35 and 3.38 (2s, 3H, **CH_3**OCH_2), 3.80 (m, 1H, **CH**), 3.81 (s, 3H, O**CH_3**), 3.88 and 4.02 (dd, d, 1H, **CH_2**O, J = 10.7, 2.2), 3.99 and 4.21 (2d, 1H, **CH_2**O, J = 10.7), 4.59 and 4.67 (2d, 1H, O**CH_2**O, J = 7.0), 4.76 and 4.78 (2d, 1H, O**CH_2**O, J = 7.0), 5.96−6.01 (m, 1H, **NH**CO), 6.45 and 6.47 (2d, 1H, **H_{arom}**, J = 8.3), 6.51 and 6.53 (2d, 1H, **H_{arom}**, J = 8.3), 7.10 (t, 1H, **H_{arom}**, J = 8.3). Anal. ($C_{16}H_{21}NO_5$) C, H, N.

3-Hydroxy-6-oxo-5′-methoxy-3′,4′-dihydrospiro[piperidine-2,3′(2′*H*)-benzopyran] (16). Compound **15** (1.000 g, 3.26 mmol) was dissolved in MeOH (8.8 mL), and Amberlyst 15, ion-exchange resin (Aldrich), was added. The mixture was stirred at room temperature. After total consumption of the

derivative **15**, Amberlyst 15, ion-exchange resin (Aldrich), was filtered and washed with MeOH. The solvent was evaporated, and column chromatography (eluent: CH_2Cl_2/MeOH, 9/1) gave the expected alcohol **16** as an amorphous solid (0.754 g) in 88% yield: IR (KBr) ν 3610−3020 (NH and OH), 1640 (C═O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.70−1.82 (m, 2H, **CH$_2$**CH), 1.89−2.15 (m, 1H, **CH$_2$**CO), 2.28−2.43 (m, 1H, **CH$_2$**CO), 2.37 and 2.50 (2d, 1H, Ar**CH$_2$**, J = 17.1), 2.75 and 3.20 (2d, 1H, Ar**CH$_2$**, J = 17.1), 3.59−3.69 (m, 2H, **CH$_2$**O, **CH**O), 3.75 (s, 3H, O**CH$_3$**), 4.01 and 4.13 (dd, d, 1H, **CH$_2$**O, J = 10.1, 1.4), 5.04 and 5.17 (2d, 1H, **OH**, J = 5.0), 6.41 (d, 1H, **H$_{arom}$**, J = 8.3), 6.54 (d, 1H, **H$_{arom}$**, J = 8.3), 7.04 (t, 1H, **H$_{arom}$**, J = 8.3), 7.11 (s, 1H, **NH**); MS (CI/NH$_3$) m/z 264 (M + 1). Anal. ($C_{14}H_{17}NO_4$) C, H, N.

3-[(Imidazolylthiocarbonyl)oxy]-6-oxo-5′-methoxy-3′,4′-dihydrospiro[piperidine-2,3′(2′*H*)-benzopyran] (17). Alcohol **16** (0.380 g, 1.44 mmol) in dry THF (5 mL) and *N*,*N*-thiocarbonyldiimidazole (0.644 g, 3.61 mmol) were refluxed for 4 h. After hydrolyzing, the product was extracted with CH_2Cl_2. A column chromatography purification (eluent: CH_2Cl_2/MeOH, 9/1) afforded the desired compound **17** as a powder (0.500 g) in 93% yield: mp 100−110 °C dec; IR (KBr) ν 1660 (C═O), 1230 (C−O−C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.27−2.37 (m, 1H, **CH$_2$**CH), 2.42−2.52 (m, 2H, **CH$_2$**CH, **CH$_2$**CO), 2.55−2.63 (m, 1H, **CH$_2$**CO), 2.86 (d, 1H, Ar**CH$_2$**, J = 16.9), 3.00 (d, 1H, Ar**CH$_2$**, J = 16.9), 3.78 and 3.83 (2s, 3H, O**CH$_3$**), 3.09 (d, 1H, **CH$_2$**O, J = 10.5), 4.07 (d, 1H, **CH$_2$**O, J = 10.5), 5.87−5.92 (m, 1H, **CH**O), 5.97 (br s, 1H, **NH**), 6.47 (d, 1H, **H$_{arom}$**, J = 8.3), 6.55 (d, 1H, **H$_{arom}$**, J = 8.3), 7.08 (s, 1H, **CH**N), 7.14 (t, 1H, **H$_{arom}$**, J = 8.3), 7.59 (s, 1H, **CH**N), 8.34 (s, 1H, **CH**N); MS (CI/NH$_3$) m/z 374 (M + 1). Anal. ($C_{18}H_{19}N_3O_4S$) C, H, N, S.

6-Oxo-5′-methoxy-3′,4′-dihydrospiro[piperidine-2,3′-(2′*H*)-benzopyran] (18). Tributyltin hydride (0.750 g, 2.57 mmol) in dry toluene (8 mL) was refluxed. A solution of compound **17** (0.480 g, 1.29 mmol) in a mixture THF/toluene (1/2) (8 mL) was added dropwise to maintain the tributyltin hydride solution at reflux. Then a catalytic amount of 2,2′-azobis(2-methylpropionitrile) was added. The mixture was heated at reflux. After total consumption of starting material, the mixture was hydrolyzed. The product was extracted with CH_2Cl_2 and purified by column chromatography (eluent: CH_2Cl_2/MeOH, 9/1) to give the expected compound **18** as a powder (0.300 g) in 94% yield: mp 140−141 °C; IR (KBr) ν 3190 (NH), 1670 (C═O), 1240 (C−O−C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.72−1.78 (m, 2H, **CH$_2$**), 1.88−2.04 (m, 2H, **CH$_2$**), 2.42 (t, 2H, CO**CH$_2$**, J = 6.6), 2.69 (d, 1H, Ar**CH$_2$**, J = 16.9), 2.82 (dd, 1H, Ar**CH$_2$**, J = 16.9, 2.0), 3.80 (s, 3H, O**CH$_3$**), 3.85 (d, 1H, **CH$_2$**O, J = 10.6), 3.92 (dd, 1H, **CH$_2$**O, J = 10.6, 2.0), 6.04 (br s, 1H, **NH**), 6.47 (d, 1H, **H$_{arom}$**, J = 8.3), 6.53 (d, 1H, **H$_{arom}$**, J = 8.3), 7.10 (t, 1H, **H$_{arom}$**, J = 8.3). Anal. ($C_{14}H_{17}NO_3$) C, H, N.

5′-Methoxy-3′,4′-dihydrospiro[piperidine-2,3′(2′*H*)-benzopyran] (19). Derivative **19** was prepared according to the method used for **6a** from compound **18** (0.300 g, 1.21 mmol). The expected amine **19** was given, after column chromatography (eluent: CH_2Cl_2/MeOH, 9/1), as an oil (0.200 g) in 71% yield: IR (film) ν 3740−3040 (NH), 1235 (C−O−C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.47−1.72 (m, 352H, 35**CH$_2$**), 1.77 (br s, 1H, **NH**), 2.52 (d, 1H, Ar**CH$_2$**, J = 17.1), 2.83 (dd, 1H, Ar**CH$_2$**, J = 17.1, 2.0), 2.87 (t, 2H, **CH$_2$**N, J = 5.3), 3.81 (s, 3H, O**CH$_3$**), 3.81 (d, 1H, **CH$_2$**O, J = 10.7), 4.05 (dd, 1H, **CH$_2$**O, J = 10.7, 2.0), 6.51 (d, 1H, **H$_{arom}$**, J = 8.3), 6.43 (d, 1H, **H$_{arom}$**, J = 8.3), 7.07 (t, 1H, **H$_{arom}$**, J = 8.3); MS (CI/NH$_3$) m/z 234 (M + 1). Anal. ($C_{14}H_{19}NO_2$) C, H, N.

1-(4-Aminobutyl)-5′-methoxy-3′,4′-dihydrospiro[piperidine-2,3′(2′*H*)-benzopyran] (21). To a solution of amine **19** (0.320 g, 1.37 mmol) in dry DMF (6 mL) were added potassium carbonate (0.948 g, 6.87 mmol) and 4-bromobutyronitrile (1.016 g, 6.87 mmol). The mixture was heated at 60 °C for 24 h, DMF was removed by evaporation, and the residue was hydrolyzed. The crude product was extracted with CH_2Cl_2. After purification by chromatography (eluent: CH_2Cl_2/MeOH, 95/5), the expected compound **20** was obtained as an oil (0.380 g) in 92% yield. Then, to a suspension of lithium aluminum hydride (0.028 g, 0.73 mmol) in dry ether (Et_2O) (3 mL) was added dropwise a solution of **20** (0.110 g, 0.37 mmol) in Et_2O at 0 °C. After refluxing for 2 h, the mixture was allowed to cool to 0 °C. Then, successively, water (0.028 mL), 15% sodium hydroxide solution (0.028 mL), and water (0.084 mL) were added very slowly. After stirring for 15 min at 0 °C, the salts were filtered and washed with ether. The solvent was removed to give the expected amine **21** as an oil (0.112 g) in quantitative yield. An analytical sample was obtained after column chromatography (eluent: CH_2Cl_2/MeOH, 9/1): IR (film) ν 3680−3100 (NH$_2$), 1235 (C−O−C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.38−1.64 (m, 12H, **CH$_2$**, **NH$_2$**), 2.30−2.43 (m, 1H, **CH$_2$**), 2.46−2.55 (m, 1H, **CH$_2$**), 2.58−2.78 (m, 6H, **CH$_2$**NH$_2$, Ar**CH$_2$**, **CH$_2$**), 3.82 (s, 3H, O**CH$_3$**), 3.91 (dd, 1H, **CH$_2$**O, J = 10.4, 1.0), 4.02 (d, 1H, **CH$_2$**O, J = 10.4), 6.43 (d, 1H, **H$_{arom}$**, J = 8.2), 6.48 (d, 1H, **H$_{arom}$**, J = 8.2), 7.04 (t, 1H, **H$_{arom}$**, J = 8.2). Anal. ($C_{18}H_{28}N_2O_2$) C, H, N.

1-*n*-Propyl-5′-methoxy-3′,4′-dihydrospiro[piperidine-2,3′(2′*H*)-benzopyran] (22). Amine **19** (0.125 g, 0.54 mmol) in dry acetonitrile (CH_3CN) (6 mL), potassium carbonate (0.518 g, 3.75 mmol), 1-iodopropane (0.320 g, 1.88 mmol), and potassium iodide (catalytic amount) were refluxed. After total consumption of starting material, the mixture was hydrolyzed and the crude product was extracted with CH_2Cl_2. After column chromatography (eluent: CH_2Cl_2/MeOH, 9/1), the expected compound **22** was obtained as an oil (0.110 g) in 75% yield: IR (film) ν 1245 (C−O−C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, **CH$_3$**, J = 7.3), 1.34−1.68 (m, 452H, 45**CH$_2$**), 2.27−2.39 (m, 1H, **CH$_2$**CH$_2$CH$_3$), 2.42−2.53 (m, 1H, **CH$_2$**CH$_2$CH$_3$), 2.59−2.81 (m, 4H, Ar**CH$_2$**, **CH$_2$**N), 3.83 (s, 3H, O**CH$_3$**), 3.93 (d, 1H, **CH$_2$**O, J = 10.7), 4.03 (d, 1H, **CH$_2$**O, J = 10.7), 6.44 (d, 1H, **H$_{arom}$**, J = 8.2), 6.49 (d, 1H, **H$_{arom}$**, J = 8.2), 7.05 (t, 1H, **H$_{arom}$**, J = 8.2); MS (CI/NH$_3$) m/z 276 (M + 1). Anal. ($C_{17}H_{25}NO_2$) C, H, N.

1-[4-(7,9-Dioxo-8-azaspiro[4.5]decanyl)butyl]-5′-methoxy-3′,4′-dihydrospiro[piperidine-2,3′(2′*H*)-benzopyran] (23). Compound **23** was prepared according to the method used for **22** from amine **19** (0.130 g, 0.56 mmol) and 8-(4-bromobutyl)-8-azaspiro[4.5]decane-7,9-dione (0.253 g, 0.84 mmol). The crude product was chromatographed (eluent: CH_2Cl_2/MeOH, 9/1) to give the desired compound **23** as an oil (0.225 g) in 89% yield: IR (film) ν 1710 and 1655 (C═O), 1230 (C−O−C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.34−1.62 (m, 752H, 75**CH$_2$**), 1.68−1.74 (m, 252H, 25**CH$_2$**), 2.30−2.40 (m, 1H, **CH$_2$**), 2.46−2.57 (m, 1H, **CH$_2$**), 2.59 (s, 4H, **CH$_2$**CO), 2.61−2.78 (m, 4H, **CH$_2$**, Ar**CH$_2$**), 3.76 (t, 2H, **CH$_2$**, J = 7.2), 3.83 (s, 3H, O**CH$_3$**), 3.91 (dd, 1H, **CH$_2$**O, J = 10.4, 1.4), 3.98 (d, 1H, **CH$_2$**O, J = 10.4), 6.42 (d, 1H, **H$_{arom}$**, J = 8.2), 6.47 (d, 1H, **H$_{arom}$**, J = 8.2), 7.04 (t, 1H, **H$_{arom}$**, J = 8.2); MS (CI/NH$_3$) m/z 455 (M + 1). Anal. ($C_{27}H_{38}N_2O_4$) C, H, N.

1-[4-(4-Toluenesulfonamido)butyl]-5′-methoxy-3′,4′-dihydrospiro[piperidine-2,3′ (2′*H*)-benzopyran] (24). To a solution of amine **21** used without purification (0.096 g, 0.32 mmol) in dry CH_2Cl_2 (3 mL) were added dropwise at 0 °C triethylamine (0.096 g, 0.95 mmol) and *p*-toluenesulfonyl chloride (0.060 g, 0.32 mmol) in CH_2Cl_2 (2 mL). The mixture was stirred at room temperature. After 30 min, the mixture was hydrolyzed and the product was extracted with CH_2Cl_2. Column chromatography (eluent: CH_2Cl_2/MeOH, 9/1) afforded the expected compound **24** as an oil (0.115 g) in 80% yield: IR (film) ν 3400−3160 (NH), 1230 (C−O−C), 1320 and 1150 (SO$_2$) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.34−1.68 (m, 11H, **NH**, **CH$_2$**), 2.30−2.74 (m, 252H, 25**CH$_2$**), 2.42 (s, 3H, **CH$_3$**), 2.69 (s, 2H, Ar**CH$_2$**), 2.86−2.98 (m, 2H, **CH$_2$**NSO$_2$), 3.82 (s, 3H, O**CH$_3$**), 3.92 (d, 1H, **CH$_2$**O, J = 10.7), 4.02 (d, 1H, **CH$_2$**O, J = 10.7), 6.43 (d, 1H, **H$_{arom}$**, J = 8.2), 6.47 (d, 1H, **H$_{arom}$**, J = 8.2), 7.05 (t, 1H, **H$_{arom}$**, J = 8.2), 7.30 (d, 2H, **H$_{arom}$**, J = 8.1), 7.74 (d, 2H, **H$_{arom}$**, J = 8.1); MS (CI/NH$_3$) m/z 459 (M + 1). Anal. ($C_{25}H_{34}N_2O_4S$) C, H, N, S.

1-[4-(4-Fluoro-1-benzamido)butyl]-5′-methoxy-3′,4′-dihydrospiro[piperidine-2,3′(2′*H*)-benzopyran] (25). Compound **25** was prepared according to the method used for **24** from amine **21** (0.175 g, 0.58 mmol) and 4-fluorobenzoyl chloride (0.091 g, 0.58 mmol). The expected product **25** was obtained after column chromatography (eluent: CH_2Cl_2/MeOH, 9/1) as an oil (0.165 g) in 67% yield: IR (film) ν 3560−3140 (NH), 1620 (C═O), 1230 (C−O−C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.42−1.69 (m, 552H, 55**CH$_2$**), 2.38−2.49 (m, 1H, **CH$_2$**), 2.54−2.63 (m, 1H, **CH$_2$**), 2.63−2.79 (m, 2H, **CH$_2$**), 2.69 (s, 2H,

Ar**CH$_2$**), 3.42 (td, 2H, **CH$_2$**NCO, J = 6.5, 6.7), 3.83 (s, 3H, O**CH$_3$**), 3.90 (d, 1H, **CH$_2$**O, J = 10.8), 4.03 (d, 1H, **CH$_2$**O, J = 10.8), 6.14−6.22 (m, 1H, **NH**), 6.43 (d, 1H, **H$_{arom}$**, J = 8.2), 6.47 (d, 1H, **H$_{arom}$**, J = 8.2), 7.05 (t, 1H, **H$_{arom}$**, J = 8.2), 7.10 (t, 2H, **H$_{arom}$**, J = 8.5), 7.74 (d, 1H, **H$_{arom}$**, J = 8.5), 7.77 (d, 1H, **H$_{arom}$**, J = 8.5); MS (CI/NH$_3$) m/z 427 (M + 1). Anal. ($C_{25}H_{31}N_2O_3F$) C, H, N, F.

Biology. Binding Experiments. Receptor binding assays were conducted using methods previously reported in the literature.[57] Briefly, 5-HT$_{1A}$ assays used rat hippocampus membranes, [^{3}H]-8-OH-DPAT, and buspirone for nonspecific binding (NSB). 5-HT$_{1B}$ assays used rat cortex + striatum + globuspallidus, [^{3}H]-5-OH-tryptamine, and serotonin for NSB. 5-HT$_2$ assays used calf frontal cortex, [^{3}H]ketanserin, and spiperone for NSB. 5-HT$_3$ assays used NG10815 cells, [^{3}H]-BRC 36694, and ICS 205930 for NSB. D$_1$ assays used calf striatum, [^{3}H]raclopride, and haloperidol for NSB. D$_2$ assays used calf caudate nucleus, [^{3}H]SDZ205-501, and butaclamol for NSB.

The concentration of the radioligands used in competition studies were approximately equal to the K_D of the binding system. The affinity of the ligands tested to these receptors was expressed as IC$_{50}$ (concentration inhibiting 50% of the specific binding) and calculated using LUNDON 2 software. The results obtained are reported in Table 5.

Adenylate Cyclase Experiments. Activation of postsynaptic 5-HT$_{1A}$ receptors is linked to an inhibition of forskolin-activated adenylate cyclase in rat hippocampal membranes. Briefly, rat hippocampal membranes were incubated for 20 min at 30 °C with forskolin (10 μM), [α-^{32}P]ATP (0.1 μM), and the compound to be tested. The enzymatic activity was estimated from the conversion of [α-^{32}P]ATP into [^{32}P]cAMP (in nM/mg of protein/20 min at 30 °C).[58]

Tail Suspension Test in Mice. Swiss mice were suspended by the tail for 6 min. The behavior of the animals was recorded automatically using a special computerized apparatus (Itematic TST) which measured the duration of immobility and the power of movements. Compound (+)-**11a** was administered 30 min before the test at doses of 32 and 64 mg/kg ip.[59]

Behavioral Despair Test (Forced Swim Test). The animal was placed in a cylinder containing water from which it could not escape. In the mouse version (groups of 10 animals), the animal was placed for 6 min in the water and the duration of immobility during the last 4 min was measured. (+)-**11a** was administered once 30 min before the test, at doses of 32 and 64 mg/kg ip.

In the rat version of the test (groups of six animals), the animal was pre-exposed to the water for 15 min on the first day of the experiment and then put back in the water 24 h later for a 5-min test. (+)-**11a** was administered 24, 5, and 1 h before the test at doses of 16, 32, and 64 mg/kg ip.[60,61]

Light−Dark Box Test in Mice. The anxiolytic-like activity of the compounds was tested using an unconditioned conflict test, the light−dark choice procedure behaviorally validated for detecting antianxiety agents in mice.[62] In brief, the apparatus consisted of two poly(vinyl chloride) tools covered by Plexiglass. One of these boxes was darkened, and the other was lightened by a lamp. Mice were placed in the lit box to start the test session. The amount of time spent by mice in the lit box (TLB) and the number of transitions through the tunnel were reported over a 5 min period after the first entry in the dark box. A mouse with all four paws in the new box was considered as having changed boxes. The compound was tested at 5, 7.5, and 10 mg/kg. The lack of sedative effect of the compound at the tested doses was previously measured in a free exploratory test.

The statistical significance of differences between control and treated groups was ascertained by a combined analysis of variance and a Dunnett's or Bonferroni's *posteriori t*-test.

Elevated Plus Maze. Swiss mice (10/group) were introduced into the plus maze, a cruciform labyrinth consisting of two closed arms and two open ones. The exploratory behavior of the mouse was scored for 10 min according to the number of entries in the open and closed arms, as well as the time spent in the open arms. (+)-**11a** was intraperitoneally administered 30 min before the test at doses of 32 and 64 mg/kg.[63]

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Supporting Information Available: Eight tables (Tables A−H) of molecular modeling results (10 pages). Ordering information is given on any current masthead page.

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